SCANNING ELECTRON MICROSCOPY OF THE ENVELOPES SURROUNDING THE CHUM-SALMON OOCYTES

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SCANNING ELECTRON MICROSCOPY OF THE ENVELOPES SURROUNDING THE CHUM-SALMON OOCYTES[1]

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The envelopes surrounding the Chum-salmon oocytes in pre- and post-ovulation stages were observed with the scanning electron microscope. The follicle cells adhering on the chorion of the ovarian oocytes were dispersed at ovulation. The micropyle became observable in the ovulated ripe eggs.

Previously, the author and his co-workers described the process of oocyte maturation in the Chum-salmon, *Oncorrhynchus keta*, homing to the parent river (OSANAI, HIRAI and SATO 1973, OSANAI, SATO and HIRAI 1972). In the female salmons which arrived at the Otuchi Bay, Iwate Prefecture, during the months of November 1969 and 1970 the oocytes were either in the end of the growth period of oogenesis or in the beginning of the maturation period. A series of changes which proceeded in the oocytes from the time of arrival in the bay until the fish entered the Otuchi River (spawning place) was divided into four stages; A, B, C and R. Yolk was globular in Stage A. In Stage B yolk coalescence proceeded and oil drops were migrating towards the egg periphery. In the Stage C the oil drops segregated beneath the cortical cytoplasm, which formed the blastodisc at the animal pole, and the germinal vesicle disappeared. The Stage C oocytes were apparently the same as the fertilizable ripe eggs (Stage R), but were still surrounded by the follicular envelopes. During the process from Stage C to Stage R the oocytes were ovulated from the ovary to the body cavity.

The oocytes contained in the ovary were surrounded by the theca folliculi. The follicular epithelium lying between the theca folliculi and the chorion was not observed after ovulation. The dispersion of the follicle cells seemed to be necessary for egg fertility acquisition. Thus, the egg envelopes were observed with the scanning electron microscope before and after ovulation.

MATERIAL AND METHOD

The ovaries and the ovulated oocytes (the ripe eggs) of the Chum-salmons, *Oncorrhynchus keta*, collected in the Otuchi Bay, Iwate Prefecture, were fixed with

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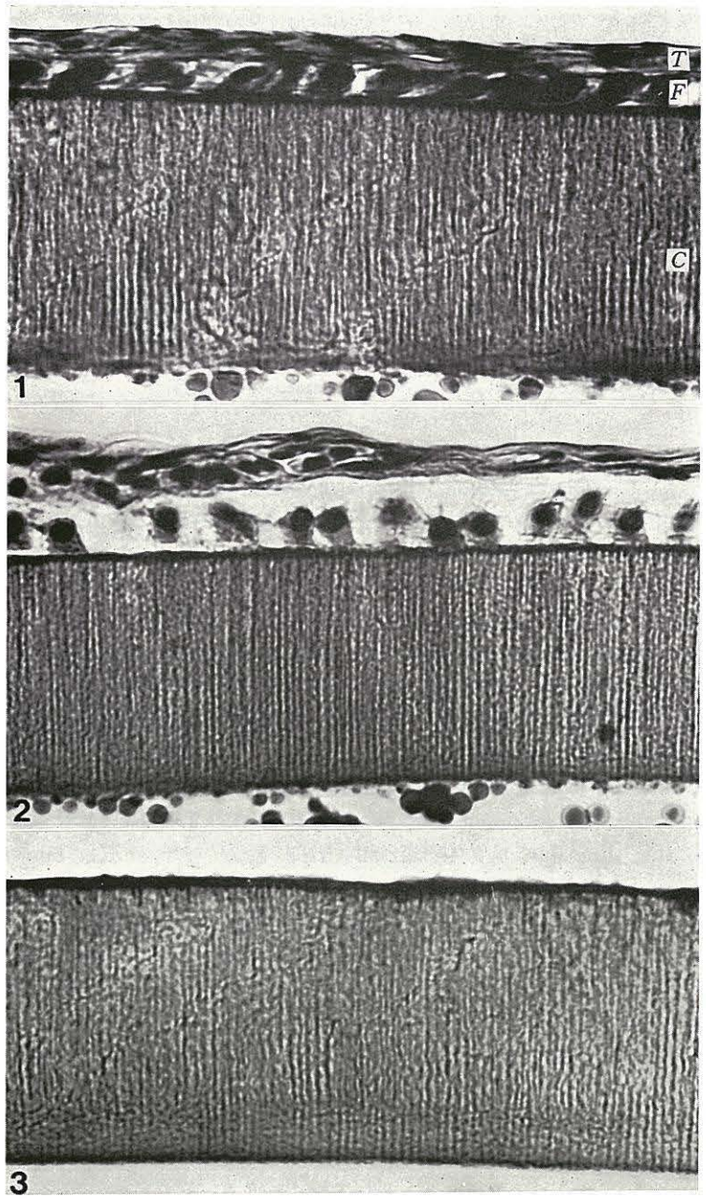

Figs. 1–3. The evnelopes of the ovarian oocytes (Figs. 1 and 2, Stage B) and the ripe egg (Fig. 3, Stage R). *C*: chorion, *F*: follicle epithelium, *T*: theca folliculi. × ca. 600 (light microscopy)

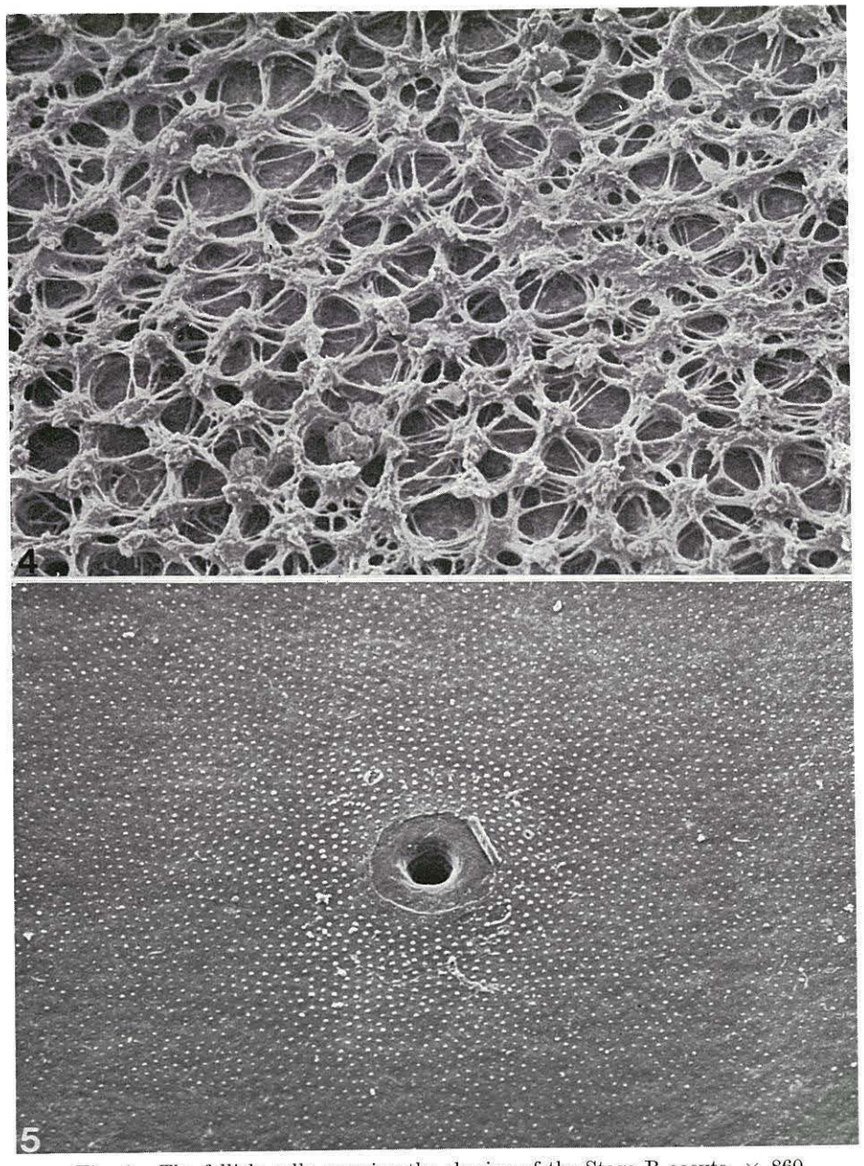

Fig. 4. The follicle cells covering the chorion of the Stage B oocyte. × 860
Fig. 5. The outer surface of the ripe egg chorion (animal pole side). × 930

Baker's formalin-calcium chloride solution. For light microscopy the ovarian oocytes enclosed with the theca folliculi and the chorions isolated from the ripe eggs were sectioned with the usual paraffin method and stained with Delafield's hematoxylin-eosin method. For scanning electron microscopy the oocytes, from

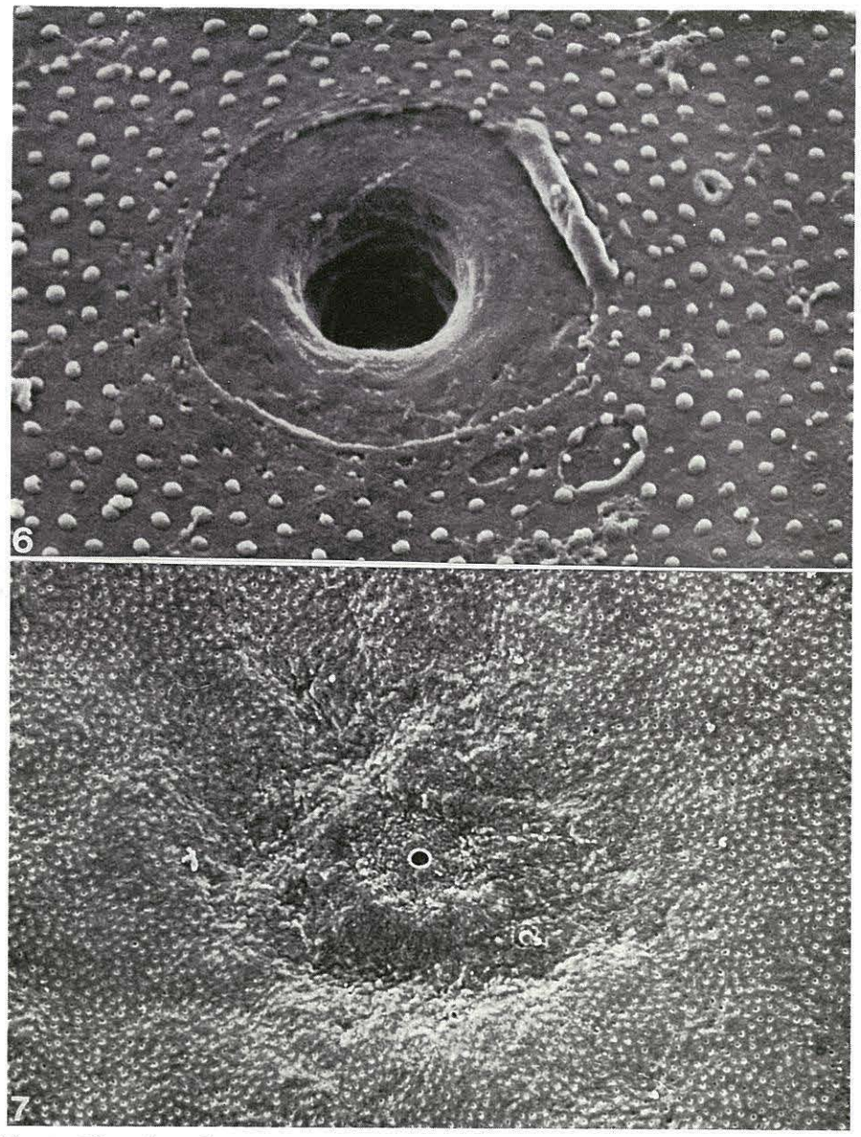

Fig. 6. The micropyle. × 3300
Fig. 7. The inner surface of the ripe egg chorion (animal pole side). The micropyle lies in the center. × 930

which the theca folliculi were stripped, and the isolated chorions of the ripe eggs were dehydrated in a series of ethyl alcohol and in acetone and then dried. The gold-carbon coated samples were examined in the scanning electron microscope, Hitachi HSM–2A.

OBSERVATION AND CONSIDERATION

The ovarian eggs were enclosed with the follicular envelopes (the theca folliculi and the follicular epithelium) (Figs. 1 and 2). After the removal of the theca folliculi the chorion was covered with the networks of the follicle cells (Fig. 4). The micropyle could not be observed with the scanning electron microscope though it was observable with the light microscope before drying.

The follicle cells were not observed on the chorion of the ovulated ripe eggs (Figs. 3 and 5). On the outer surface of the chorion the small papillae, 0.6–1 μm in diameter, were arranged regularly. At the animal pole there was the micropyle, about 8 μm in diameter, which was surrounded with the papilla-absent zone, about 5 μm in width (Figs. 5 and 6). The small holes were observed over all on the inner surface of the chorion. The holes seem to correspond to the papillae of the outer surface. The opening of the micropyle in the inner surface was narrower (about 2.7 μm in diameter) than that in the outer surface (Fig. 7).

The chorion of the ovarian oocyte was covered with the follicle cells which constituted the follicular epithelium by connecting each other's with pseudopodial processes. It seems that the connection is lossened during the later maturation stage and the follicle cells are dispersed in the body fluid at ovulation. Thus, the micropyle was opened to external media. These observations show that the dispersion of the follicle cells is a prerequsite process for egg fertility aquisition.

REFERENCES

Osanai, K., S. Hirai and R. Sato, 1973 *In vitro* induction of oocyte maturation in the Chum-salmon. Zool. Mag., **82**: 68–71. (In Japanese with English abstract)

Osanai, K., R. Sato and S. Hirai, 1972. On the maturation of the oocytes in the Chum-salmons homing to the parent river. Bull. Jap. Soc. Sci. Fish., **38**: 1247–1251. (In Japanese with English abstract)